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A METHOD FOR THE QUANTITATION OF TRACE LEVELS OF DIMETHYL SULFOXIDE IN URINE BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (U)

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C.L. Chenier and J.R. Hancock

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ABSTRACT

A semi-automated liquid chromatographic system for the extraction and concentration of trace levels of dimethyl sulfoxide (DMSO) in human urine has been developed. Urine samples are passed through a low pressure cation-exchange column as a cleanup step prior to solid phase extraction on a low pressure charcoal column. DMSO is eluted from the charcoal column with acetonitrile and after separation by reversed-phase high performance liquid chomatography, is quantified by ultra-violet detection at a wavelength of 196nm. The recovery of DMSO from the urine is 82% ± 7% (n=36) with a detection limit of 0.1 µg/mL in urine. With this method, previously unreported low levels of naturally occurring DMSO were discovered. The method also revealed a decrease in recoveries of DMSO with time indicating a potential instability of the analyte at these low concentrations.

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INTRODUCTION

According to western intelligence reports, the Eastern Bloc countries are well in the lead in research and development of chemical warfare agents (1-3). Therefore, the NATO countries are interested in designing, testing, and improving protective equipment (PE), and their associated standard operating procedures (SOPs) as a defence against these agents.

One method to evaluate the protection level of the PE and SOPs would be to use, in a realistic fashion, an intake simulant of a chemical warfare agent. Should the intake simulant enter the body and subsequently be detected in the body fluids then this would indicate faulty equipment and/or procedures.

An ideal intake simulant is a chemical that emulates the agent in all physical properties, is medically safe for absorption into the body and can be readily detected in trace amounts in body fluids.

Dimethyl sulfoxide (DMSO) has been proposed as a candidate intake simulant (4). Its physical properties are similar to those of a G-agent and it is considered to be a relatively non-toxic compound. The toxicology, pharmacology, clinical uses, fate and metabolism of DMSO in humans and animals is well documented (5-11).

Due to the high toxicity of the G-agents, it was necessary to be able to determine free DMSO concentration in the urine at levels as low as 1.0 µg/mL. The few methods that have been reported for the determination of DMSO in biological fluids (12-15), involve time consuming sample preparation and are only suitable for the determination of DMSO at relatively high concentrations (>20 µg/mL). They normally require protein precipitation either by perchloric acid or by

an organic solvent such as methanol or acetone followed by isolation and quantitation by gas chromatography.

This report describes a method capable of determining DMSO at concentrations as low as 0.1 µg/mL in urine. A semi-automated liquid chromatographic system was developed for the sample cleanup and concentration, followed by separation by reversed phase high performance liquid chromatography.

EXPERIMENTAL

Materials

Solvents and Reagents

Distilled water was used as the mobile phase on the sample preparation apparatus. For the HPLC, the distilled water was further purified daily using a commercially available photo- oxidizer (Barnstead) and then filtered through a 0.45 µM filter (Millipore). HPLC grade acetonitrile (BDH Chemicals, Omnisolve) was used for both the HPLC mobile phase and the extraction solvent on the sample preparation apparatus. Gold label DMSO (Aldrich Chemical Company) was used for the preparation of standards and spiked urine samples.

Instrumentation

A Hewlett-Packard HP1090 liquid chromatograph equipped with autosampler module and a Kratos 783 UV-VIS variable wavelength detector was used for DMSO analysis. The data was acquired and processed using a Nelson Analytical Inc. Model 6000 laboratory data system.

DMSO Spiked Urine Samples

Urine was collected from an individual over a period of four hours in order to prepare DMSO spiked urine samples. Three concentrations of DMSO in urine (1.068, 1.091, and 10.91 µg/mL) were prepared by standard addition of DMSO in water to 200 mL of urine.

The DMSO spiked urine samples were stored at room temperature in polypropylene containers in a fume hood. The urine tended to become basic with time. When necessary, HCl was added to the sample to make it acidic and thereby compatible with the ion-exchange resin. Basic samples tended to interact with the resin and form air bubbles in the column which disrupted the flow and made it impossible to collect the DMSO fraction.

Procedure

Figure 1 is a schematic of the sample preparation apparatus used for the cleanup and enrichment of urine samples.

An electronic, microprocessor-based timer (Control Model CD-4) was used to control the pumps P1 and P2 and valve V2 for reproducible and accurate heart-cutting of the DMSO from the ion-exchange column. All other valve rotations and fraction collection were performed manually.

The sample was introduced into the 4.2 mL sample loop of the low pressure sampling valve V1 (Rheodyne Model 5042 teflon rotary valve) using a 5 mL disposable plastic syringe. The valve was placed in the inject position and the timer started. This activated pump P1 (Eldex Model B-100-S) which drove the sample through the ion-exchange column

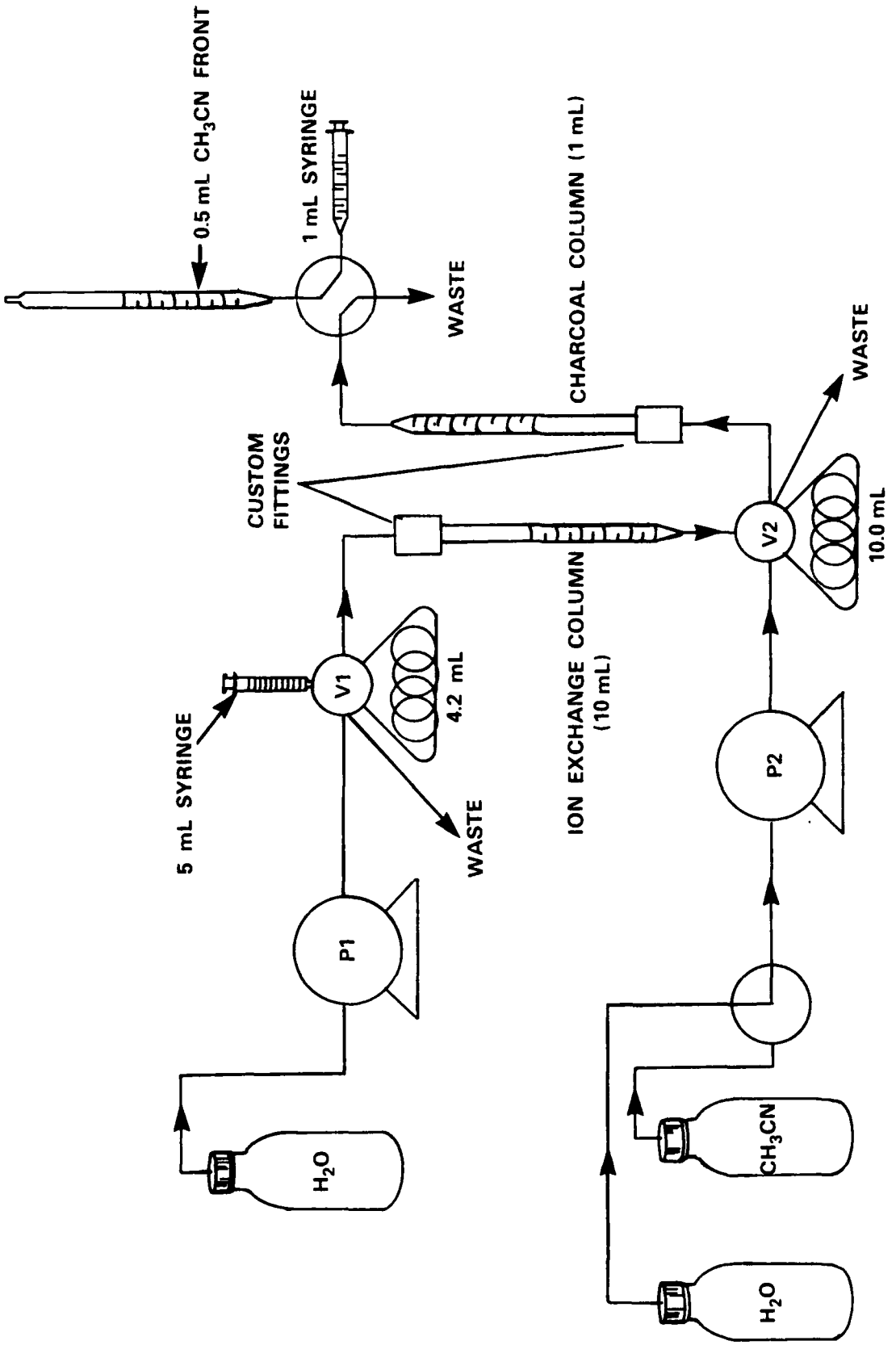

Figure 1

APPARATUS USED FOR SAMPLE PREPARATION EXPERIMENTS

at 4.25 mL/min using a 100% distilled water mobile phase. The exit of the column was attached to the 10 mL sample loop of the low pressure sampling valve V2 (Rheodyne Model 5042P) which was controlled by the timer. As soon as the DMSO fraction was in the 10 mL sample loop this valve was switched and pump P2 started. This introduced the DMSO fraction onto the charcoal column at a rate of ~2.0 mL/min, using a 100% distilled water mobile phase. As soon as the DMSO fraction was completely loaded on the charcoal column, pump P2 stopped. The flow setting was reduced to ~1.5 mL/min, the mobile phase changed to 100% acetonitrile and the pump restarted manually. A 2 mL glass pipette was used to collect the first 0.5 mL of the acetonitrile front eluting from the charcoal column. This aliquot was then drawn into a 1 mL glass syringe, and filtered (Millipore SJHV 0.45 μm) into a 2 mL autosampler vial.

Thirty six (36) aliquots from a single DMSO spiked sample (1.068 μg/mL) were used to determine the reproducibility and percent recovery of the total sample preparation method. The ion exchange column was replaced after every sixth sample while the charcoal column was replaced after every sample.

In order to determine the proper fraction to heart-cut and collect for HPLC analysis, the DMSO profiles and recovery were studied on both the ion-exchange and charcoal columns.

Ion-Exchange Column

Column Packing Procedure

Ten milliliter disposable serological pipettes (Kimble), with cotton plug were used as the columns and were packed with cation

exchange resin, AG 50W-X8, 200-400 mesh, hydrogen form (Bio-Rad Analytical Grade).

The pipette cotton plug was packed tightly into the tip using a 1/8 inch steel rod. The bulb end of the pipette was placed into an ion exchange resin slurry. Vacuum was applied until the pipette was completely filled with resin. The filled pipette was carefully placed on the clean up apparatus so that it was always full of resin and that no air entered the resin bed. A customized Swagelok fitting was used to connect the glass pipette column to the teflon tubing of the Rheodyne valve (Figure 2a). Sealing was accomplished by compression of the rubber O-ring around the glass tubing, and this method worked well under the pressures generated by the system.

DMSO Elution Profile

The DMSO elution profile for the ion-exchange column was determined by collecting 1.0 mL fractions from the outlet of the column using a manual fraction collector (a teflon block with holes drilled accurately to 1.0 ± .03 mL) and analyzing the fractions by HPLC.

Five DMSO spiked water samples of varying concentrations (1.05 to 110.0 µg/mL) were used to test the effects of concentration on the elution profile and recovery efficiency. The effect of sample matrix on the profile and recovery was also determined using a DMSO spiked urine sample of moderately high concentration (10.92 µg/mL).

Charcoal Column

Column Packing Procedure

One milliliter disposable serological borosilicate glass

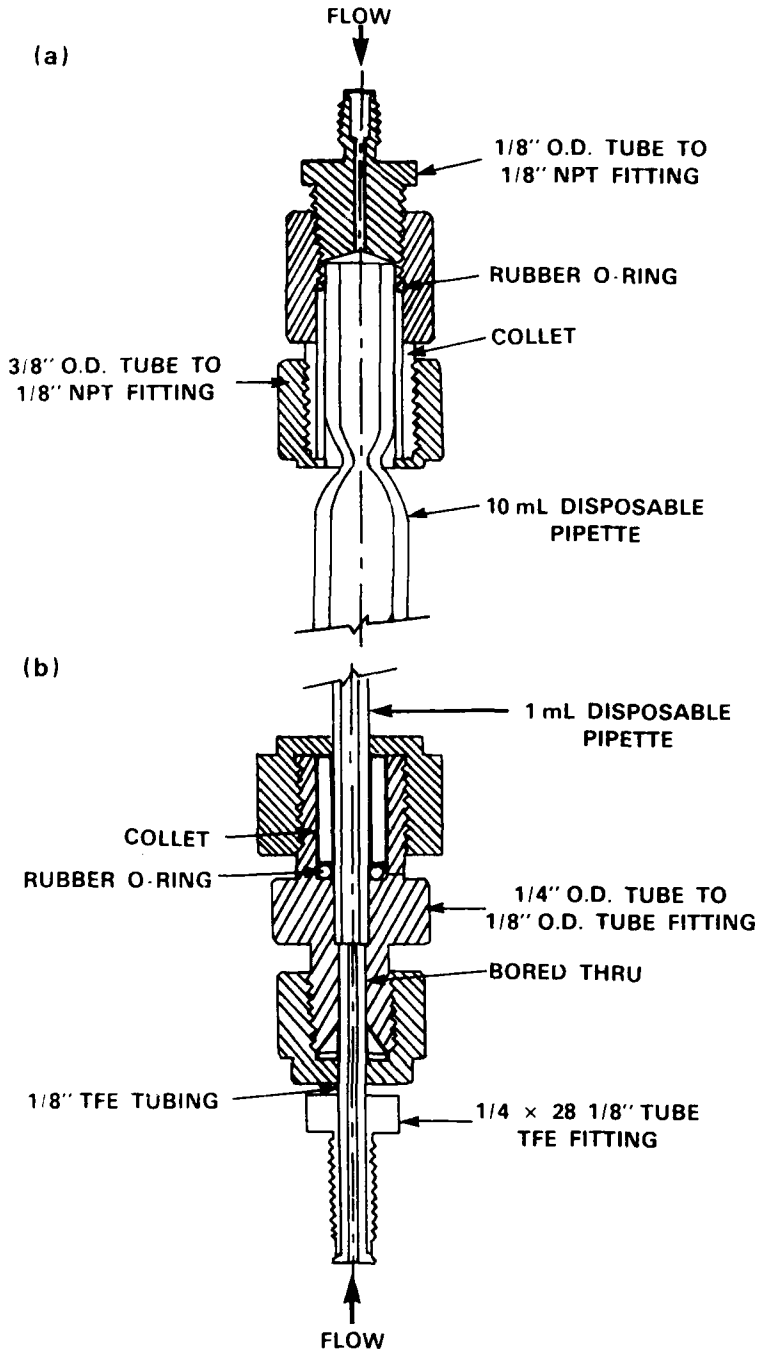

Figure 2

CUSTOM FITTINGS: a) ION-EXCHANGE COLUMN CONNECTOR, b) CHARCOAL COLUMN CONNECTOR

pipettes (Kimble) were used as columns and were packed with activated carbon, 50-200 mesh (Fisher Scientific).

The cotton plug in the pipette was packed tightly into the tip using a 1/8 inch steel rod. The pipette was completely filled with carbon and packed using a laboratory vibrator (Mettler). The carbon was held in place with a second cotton plug.

A customized Swagelok fitting was used to join this glass pipette column to the teflon tubing of the Rheodyne valve (Figure 2b). This fitting sealed well under the pressures generated by the system.

DMSO Elution Profile

The elution profile for the charcoal column was determined using DMSO spiked water samples. Four DMSO spiked water samples of varying concentrations (0.53 to 35.4 µg/mL) were used to test the effects of concentration on the elution profile and recovery efficiency. The sample was manually loaded into the sample loop of the charcoal column and injected under the normal sample preparation conditions. As soon as the acetonitrile elution was started, 0.5 mL fractions were collected from the outlet of the charcoal column using a manual fraction collector (a teflon block with holes drilled accurately to 0.5 ± .03 mL). The fractions were then analysed for DMSO content by HPLC.

RESULTS AND DISCUSSION

Ion-Exchange Column

The DMSO elution profile from the ion-exchange column was characterized using DMSO spiked water samples. Figure 3 illustrates a

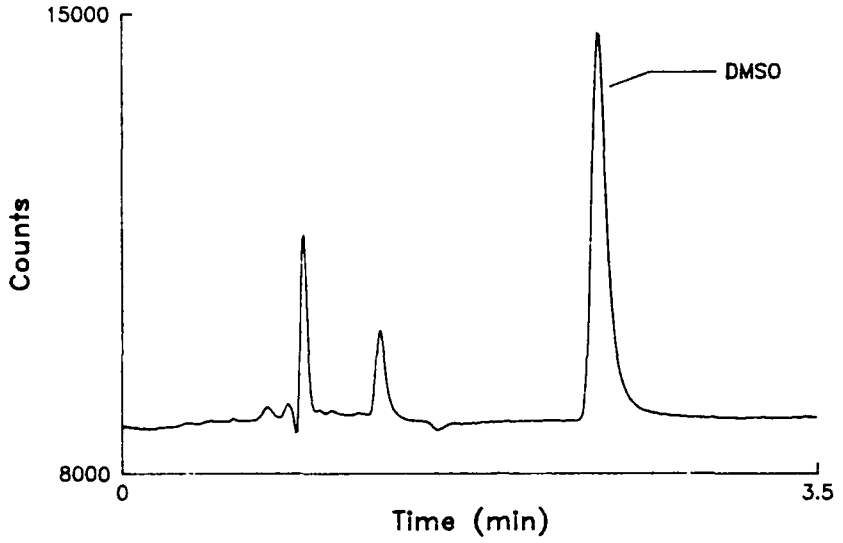

Figure 3 – HPLC chromatogram of fraction #19 from the ion exchange column using a DMSO spiked water sample. HPLC conditions – 100% distilled water; 2.0 mL/min; 25 cm Supelco RP–C18 column; 60°C; UV detection at λ = 196 nm; 10 μL injection. Retention time for DMSO is 2.37 min.

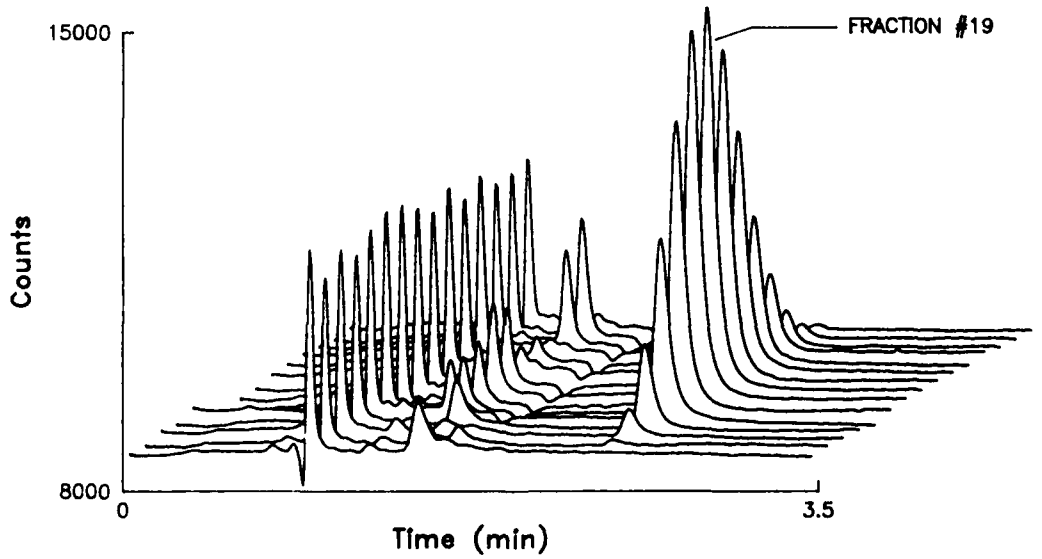

Figure 4 – A pseudo 3–dimensional plot of the DMSO profile for a DMSO spiked water sample eluting from the ion exchange column. Z–axis is fractions #12 to 26. Retention time of DMSO is 2.37 min.

typical chromatogram of a DMSO containing fraction collected from the ion-exchange column. In total, 28 fractions of 1 mL each were collected and Figure 4 shows a pseudo 3-dimensional plot of the chromatograms of fractions 12 to 26. DMSO eluted in fractions 13 to 26 and the elution profile for this compound was symmetrical and centered on fraction 19.

As only 10 mL was heart-cut from the ion-exchange column effluent for introduction onto the charcoal column, it was necessary to test for any changes in the elution profile due to DMSO concentration. Figure 5 compares the elution profiles of 3 water samples of differing DMSO concentration. In each sample the DMSO eluted in fractions 13 to 26, and it was concluded that the elution profile was not DMSO concentration dependent. Integration of the area under these curves enabled recovery efficiency to be calculated. From the elution profiles of all five available water samples the average DMSO recovery was calculated to be 94% with a relative standard deviation of 3%. Therefore the recovery was also independent of the concentration. The most concentrated sample (110.0 µg DMSO/mL H_2O) was run in triplicate to determine the reproducibility of the method on the ion-exchange column and gave a relative standard deviation for the area of 1.2% (Figure 6).

In order to determine if the elution profile would be altered by the complexity of the urine matrix, a DMSO spiked urine sample was prepared and an elution profile was obtained using the same procedure as for the DMSO spiked water samples. Figure 7 illustrates a HPLC chromatogram of one DMSO containing fraction (#19) acquired from the ion-exchange column using a DMSO spiked urine sample. Many other UV adsorbing constituents were observed but DMSO was well separated from these potential interferences. A pseudo 3-dimensional profile is shown

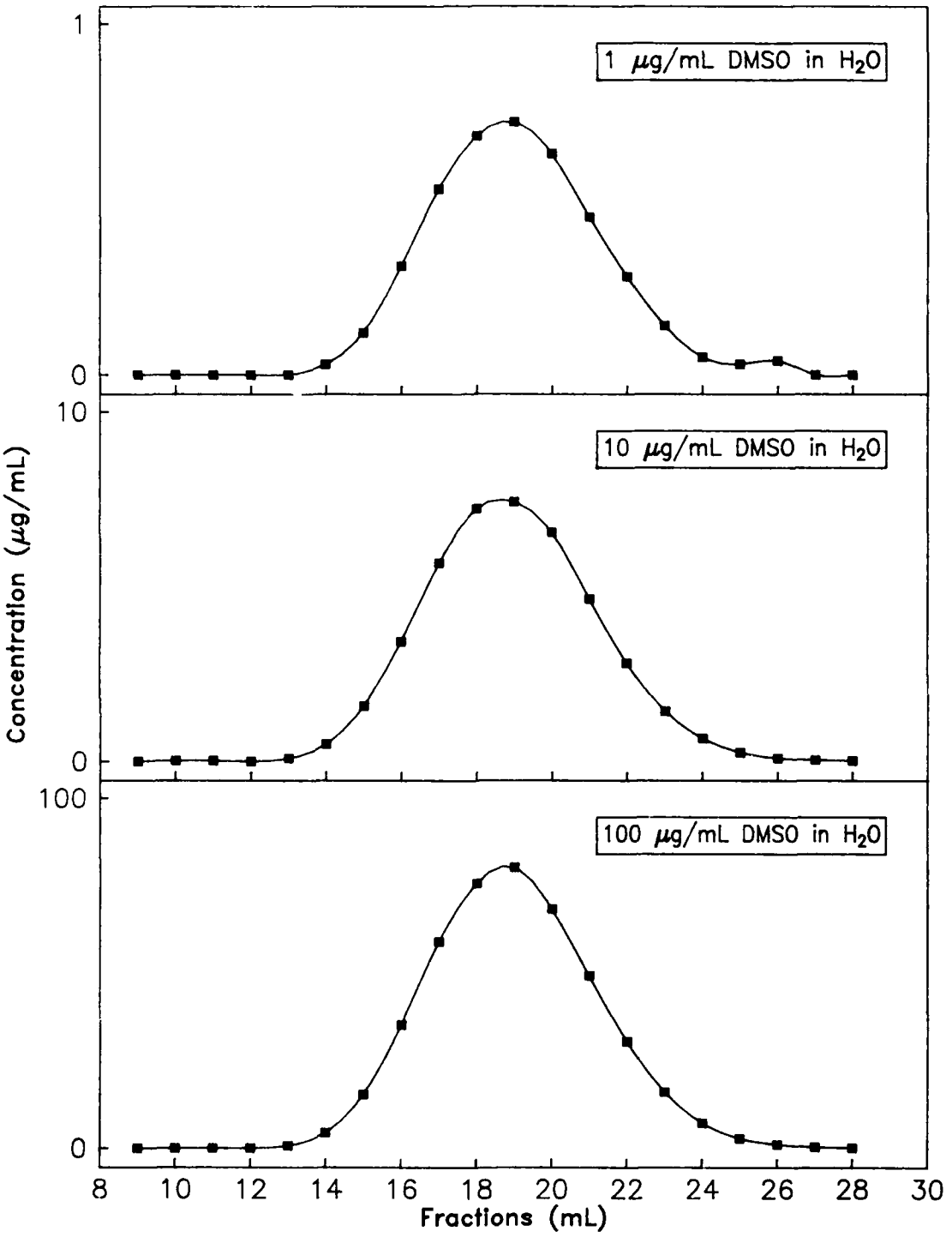

Figure 5 – Comparison of ion–exchange column elution profiles for DMSO spiked water samples of varying concentrations. 1 mL fractions were collected and analysed under the following HPLC conditions – 100% distilled water at 2.0 mL/min, 60°C, Supelco RP–C18 25 cm. UV detection at λ = 196 nm.

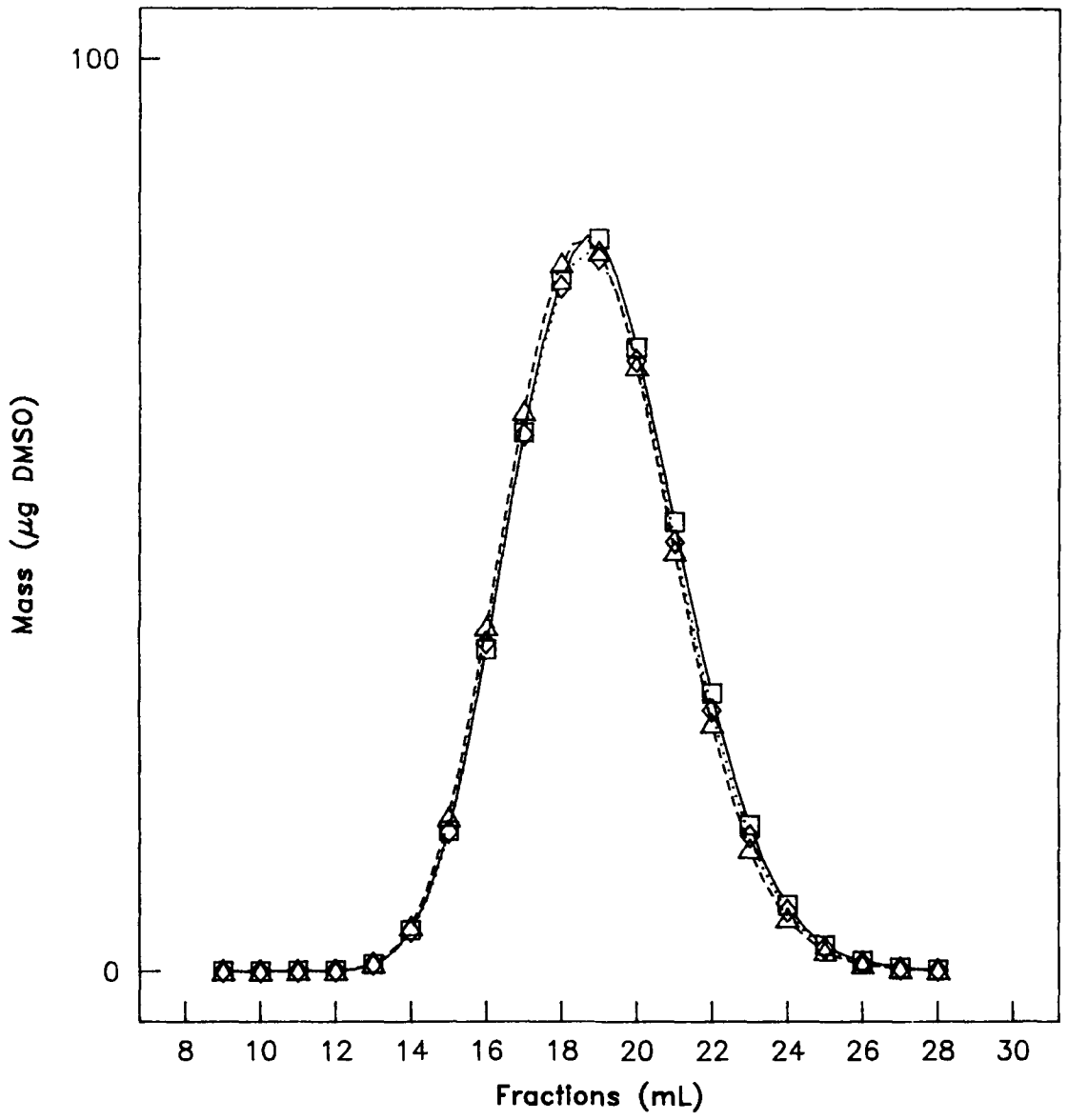

Figure 6 – Recovery profiles using the 110 μg/mL DMSO in water sample in triplicate. Relative standard deviation for the area under the curve is 1.2%.

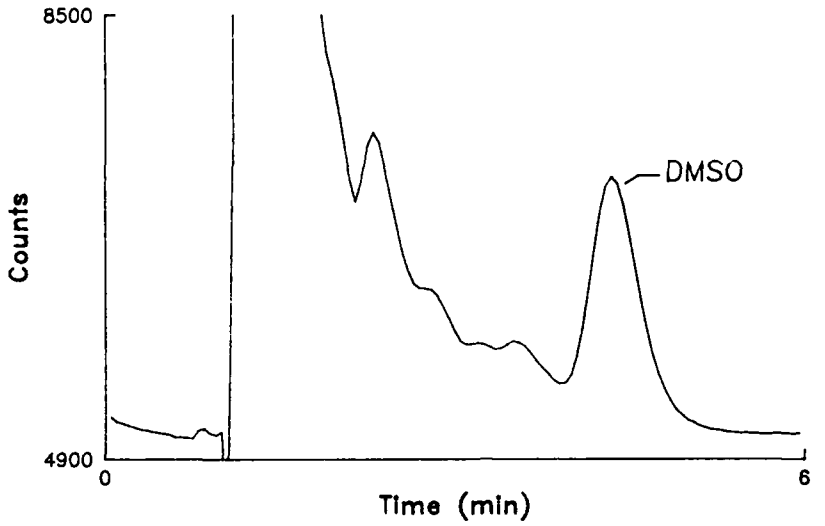

Figure 7 – HPLC chromatogram of ion–exchange column fraction #19 using a DMSO spiked urine sample. HPLC conditions – 100% distilled water; 1.0 mL/min; 6 cm Dupont Golden Series RP–C18 column; 50°C; UV detection at λ = 196; 10 μL injection. Retention time for DMSO is 4.3 min.

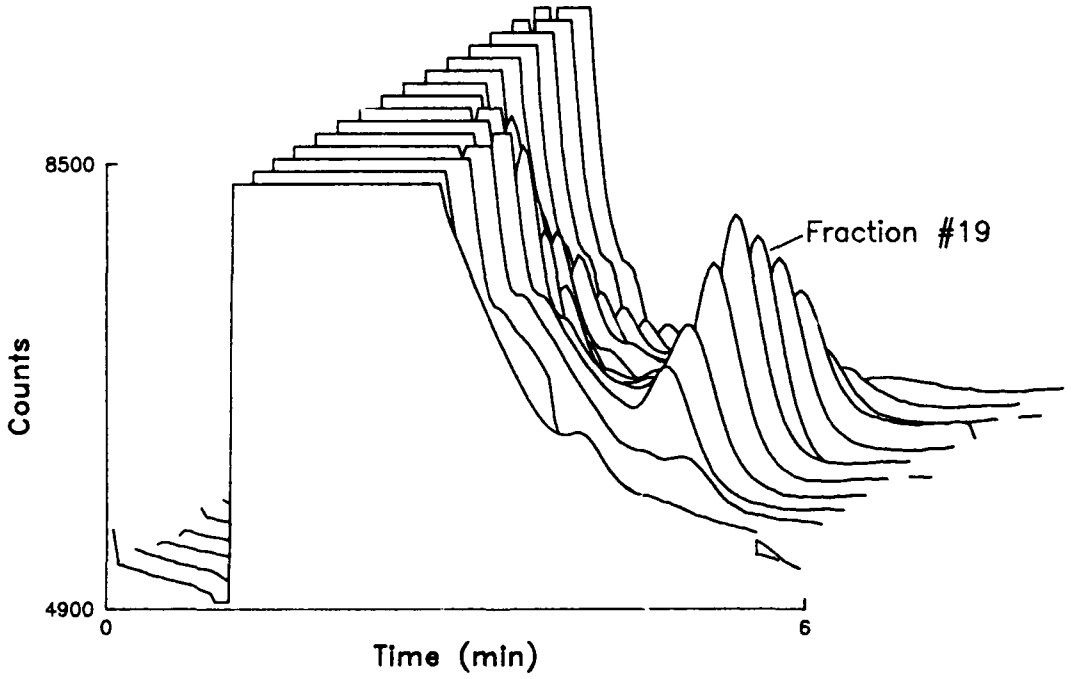

Figure 8 – A pseudo 3–dimensional plot of the DMSO profile for a DMSO in urine sample eluting from the ion–exchange column. Z–axis is fractions #12 to 26. Retention time for DMSO is 4.3 min.

in Figure 8. Although more complicated than the DMSO spiked water profile, the spiked urine profile remains symmetrical with the DMSO eluting between fractions 13 to 26. This indicates that the DMSO elution profile from the ion-exchange column was independent of sample matrix.

Charcoal Column

The elution profile of DMSO from the charcoal column was determined using DMSO spiked water samples. The pseudo 3-dimensional plot of Figure 9 shows that the DMSO elutes in a relatively sharp band between fractions 3 and 6 with the bulk in fraction 4. During the collection of the fractions it was observed that the last two to three drops of fraction 3 for the first three DMSO spiked water samples was actually acetonitrile. In the fourth DMSO spiked water sample, the acetonitrile front started in fraction 4. This was consistent with the data found in Table I indicating that the DMSO eluted in the acetonitrile front. Table I also shows that the DMSO recovery and elution profile were not concentration dependant.

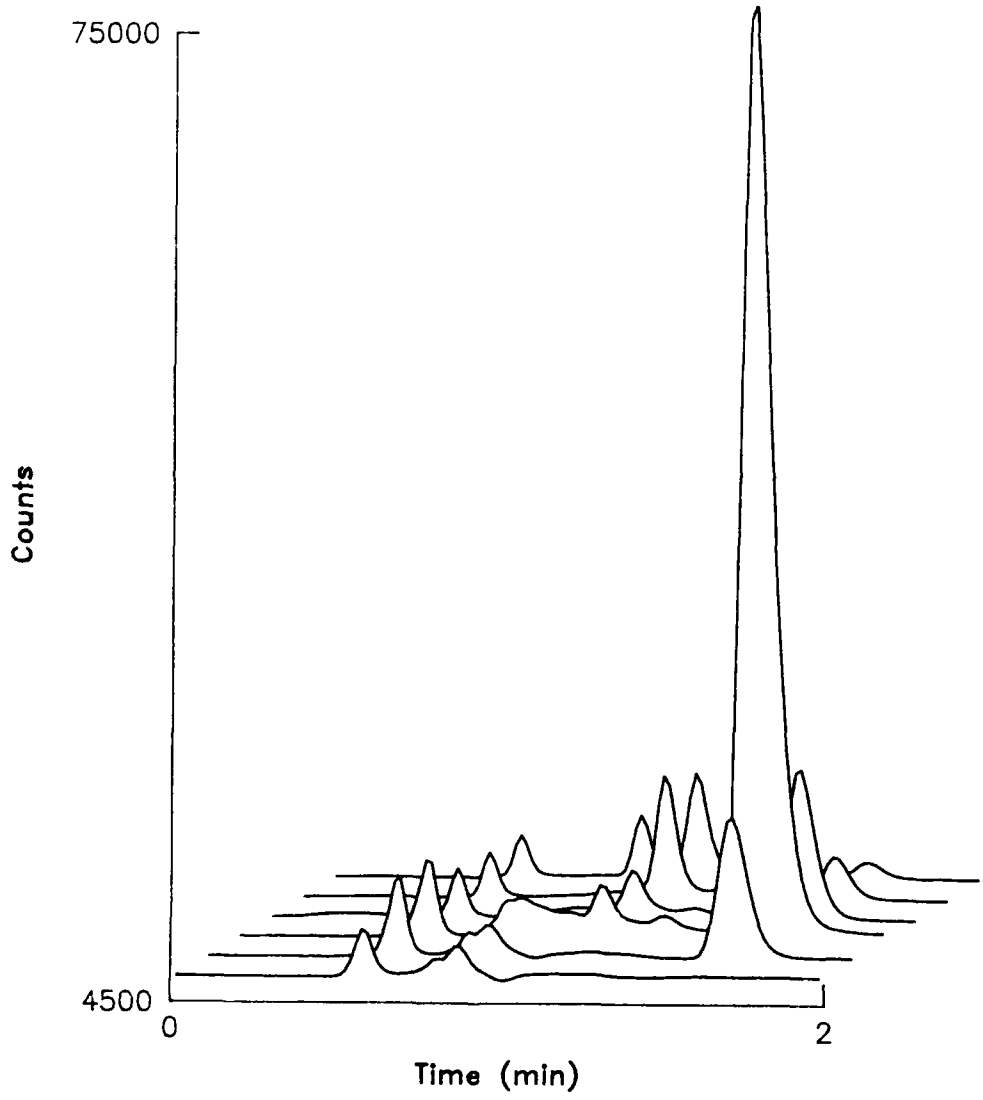

Figure 9 – A pseudo 3–dimensional plot of the DMSO profile for a DMSO spiked water sample eluting from the charcoal column. Z–axis is fractions #2 to 7. Retention time for DMSO is 1.52 min.

TABLE I

PERCENT OF TOTAL DMSO RECOVERED IN EACH FRACTION OFF THE CHARCOAL COLUMN

FRACTIONS (0.5 mL)	SAMPLE (µg/mL)			
	0.53	2.19	8.65	35.4
2	0	0	0	0
3	12	8	12	0
4	75	79	72	87
5	10	9	12	10
6	3	3	3	2
7	0	1	1	1

Sample Preparation Apparatus

HPLC Calibration Curves

In HPLC, the resolution and peak shape of the eluant can be adversely affected by the solvent strength of the injected sample (14). Since the solvent composition of the final fraction obtained from the sample preparation apparatus varied from 0 to 40% distilled water in acetonitrile, it was necessary to determine the variability of the retention time and detector response to DMSO in that solvent composition range. Variability of the solvent composition was dependent on the ability of the operator to reproducibly turn the manual valve at the appropriate time to collect the first 0.5 mL of the acetonitrile front eluting from the charcoal column.

Standard samples were prepared by the serial dilution method in three different solvents: 100% distilled water, 100% HPLC grade acetonitrile, and 50/50 distilled water/HPLC grade acetonitrile. These standards were then analyzed by HPLC. The calibration curves in Figures 10 and 11 for the three different solvents showed no significant difference in response and minimal retention time shift respectively.

System Recovery and Precision

Urine was spiked with DMSO to a concentration of 1.068 µg/mL and prepared on the sample preparation apparatus. The filtered samples from the sample preparation apparatus were analyzed quantitatively for DMSO by HPLC. Figure 12 is a chromatogram of the final fraction from the sample preparation apparatus. Under the given conditions, DMSO had a retention time of 4.65 minutes and was adequately resolved for quantitative purposes. The mass of DMSO recovered from the system was 3.84 µg which corresponded to 86% of the total DMSO in the 4.2 mL sample that was introduced onto the apparatus. By extrapolation the detection limit for DMSO in urine was determined to be 0.1 µg/mL at a signal to noise ratio of 6 to 1. Though many other peaks are present, no significant degradation of the HPLC column was observed even after analysis of more then 2000 urine samples.

Six urine samples could be passed through one ion-exchange column without significant loss of resolution of the DMSO peak (Figure 13). For this reason the ion-exchange column was replaced after every sixth sample.

A total of 36 aliquots of DMSO spiked urine (1.068 µg/mL) sample was processed on the sample preparation apparatus to determine the reproducibility and recovery of the system and the results are

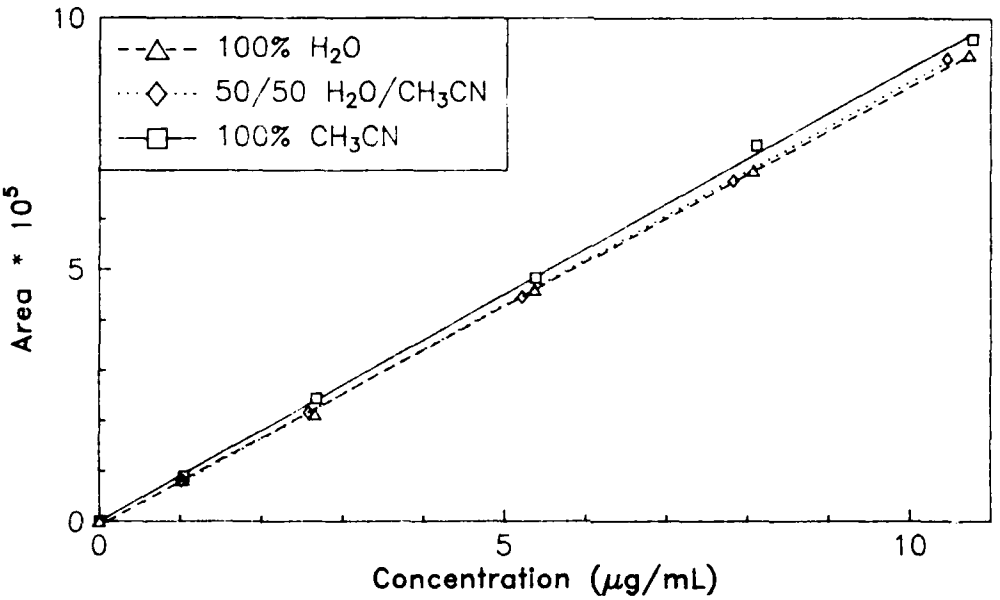

Figure 10 – Comparison of Area vs Concentration curves for DMSO in three different solvents.

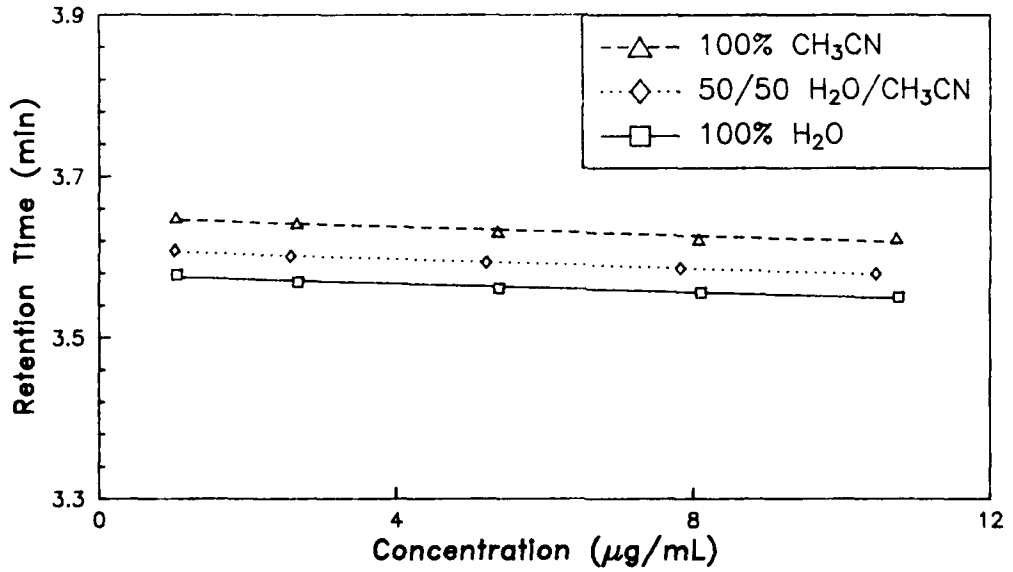

Figure 11 – Influence of solvent composition on the retention time of DMSO.

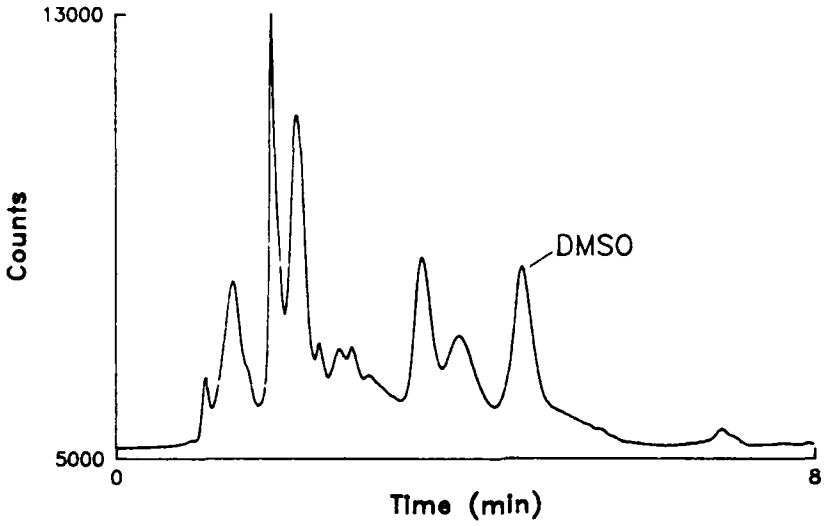

Figure 12 – HPLC chromatogram of a DMSO spiked urine sample. HPLC conditions – Dupont Zorbax Golden Series RP–C18 column; 96% acetonitrile, 4% distilled water mobile phase; 1.0 mL/min; 40°C; λ = 196 nm; 5 μL injection; DMSO retention time is 4.65 min.

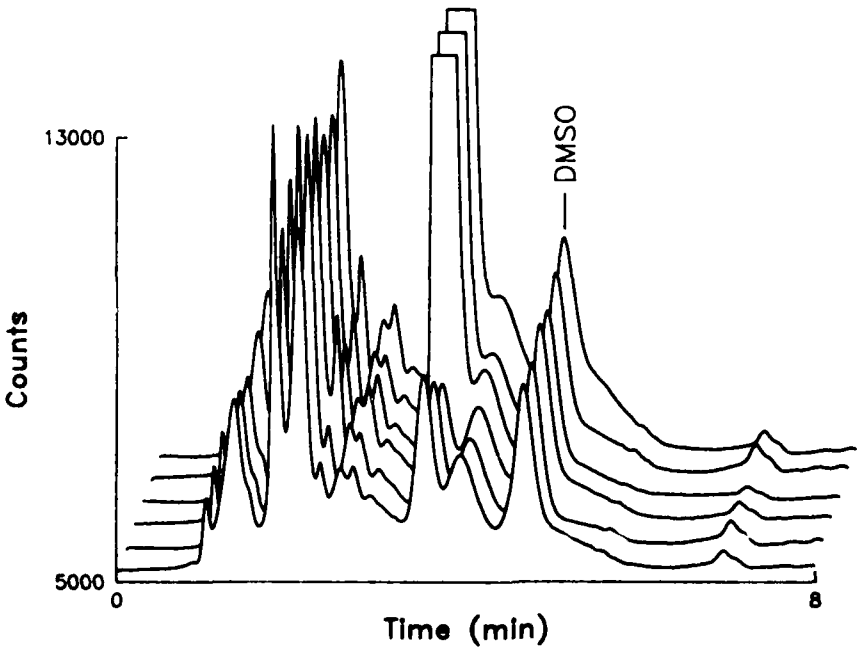

Figure 13 – Comparison of six DMSO in urine samples run through one ion exchange column. Z–axis is samples 1 to 6.

was processed on the sample preparation apparatus to determine the reproducibility and recovery of the system and the results are summarized in Table II.

TABLE II
SYSTEM REPRODUCIBILITY AND RECOVERY

Ion-Exchange Column	Average Concentration of 6 Replicates (µg/mL)	Recovery
1	7.70 ± 2.53%	86%
2	7.84 ± 2.38%	87%
3	7.22 ± 5.11%	80%
4	7.14 ± 3.65%	80%
5	7.55 ± 4.96%	84%
6	6.54 ± 5.03%	73%
		mean 82% ± 7%

The recovery was calculated using the following equation:

$$\text{Recovery} = \frac{C_A V_A}{C_u V_u} * 100\% \qquad [1]$$

Where:

C_A= concentration of the processed sample in µg/mL
V_A= volume of the processed sample = 0.5 mL
C_U= concentration of urine sample = 1.068 µg/mL
V_U= volume or urine sample = 4.2 mL

The variation of the apparatus was 7% with a combined precent recovery of 82% for a 'fresh' DMSO spiked urine sample.

Dietary DMSO in Urine Blanks

During development of the method for DMSO in urine, low levels of DMSO were detected in the blank urine samples. This could be attributed to dietary sources, as a variety of foods contain DMSO, as do some beverages including milk (15, 16).

Stability of DMSO in Urine

There is some question as to the stability of DMSO in urine. Considering that the urine is a complex matrix of organic and inorganic materials (17) it is possible that the concentration of the "free" DMSO could be reduced over time either by some chemical or biological degradation or by becoming physically bound to other constituents in the sample. To test this theory, an "old" DMSO urinary sample (spiked with DMSO 52 days prior to sample preparation and stored at room temperature) was prepared and analyzed concurrently with the "fresh" DMSO urinary samples. This "old" sample yielded in DMSO recovery of 65% as compared to 82% for the "fresh" samples. Th · icates some DMSO loss with time. A decrease in recovery was also observed in samples (Table II) which were run over a period of only three days (87% on day 1 to 73% on day 3).

The DMSO spiked urine sample which was used to determine the elution profile of the ion exchange column produced a 73% recovery of DMSO. This could also be an indication of DMSO loss as this sample was prepared on the ion-exchange column 9 days after being spiked with DMSO.

CONCLUSIONS

A method, based on solid phase extraction and concentration was developed for the quantitative determination of trace amounts of dimethyl sulfoxide (DMSO) in urine. The detection limit for DMSO in urine was 0.1 µg/mL with a recovery of 82% ± 7%.

The discovery of naturally occurring DMSO in urine would make the determination of exposure to DMSO from external sources inconclusive. One method to alleviate this problem would be to determine the rate at which the naturally occurring DMSO entered the urine and assuming that it was constant, any significant increase to this rate would indicate a DMSO intake from an external source.

The storage and handling of urine samples must be investigated further as there was some indication of DMSO loss with time. This could prove to be a significant problem in data interpretation from samples analyzed for trials, especially if the samples were stored for prolonged periods and/or degraded at different rates.

REFERENCES

1. Ingo Urban, Ostblick, (East View), October 1985, pp 15-19, ISSN: 0177-0128

2. NBC Defence and Technology International Yearbook, Vol. 2, No. 1, NBC Defence International, Ltd., New York, N.Y., 1987.

3. Jane's Defence Weekly, Vol. 7, No. 4, January 31, 1987.

4. Lockwood, P.A., personnel communication.

5. Leake, C.D., (ed.) "Biological Actions of DMSO", Ann. NY Acad. Sci., 141, pp 1-671 (1967).

6. Jacob, S.W., Rosenbaum, E.E., Wood, D.C. (ed.), "Dimethyl Sulfoxide", Vol. 1., Basic Concepts of DMSO, 1971, Marcel Dekker, Inc., New York.

7. Jacob, S.W. and Herschler, R., (ed.), "Biological Actions of DMSO", Ann. NY Acad. Sci., 243, pp 1-508 (1975).

8. de la Torre, J.C., (ed.) "Biological Actions and Medical Applications of Dimethyl Sulfoxide", Ann. NY Acad. Sci., 411, pp 1-402 (1983)

9. McCann, J., Choi, E., Yamasaki, E., and Ames, B.N., "Detection of Carcinogens as Mutagens in the Salmonella/Microsome Test: Assay of 300 Chemical Substances", Proc. Natl. Acad. Sci., U.S.A., 72, pp 5135 (1975).

10. Lobs, K., Damerau, W., and Schramm, T., "Carcinogenic Action of Dimethyl Sulfoxide", Arch. Gischwulstforsch., 37, pp 1 (1971). Chemical Abstract 75:3014 (s) (1971).

11. Survey of Compounds Which Have Been Tested for Carcinogenic Activity, United States Public Health Service Publication #149, 1972-73 (cited in 10).

12. Garretson, S.E., Aitchison, J.P., "Determination of Dimethyl Sulfoxide in Serum and Other Body Fluids by Gas Chromatography", Journal of Analytical Toxicology. Vol. 6, March/April, 1982, pp 76-81.

13. Mehta, A.C., Peaker, S., Acomb, C., Calvert, R.T., "Rapid Gas Chromatographic Determination of Dimethyl Sulfoxide and its Metabolite Dimethyl Sulphone in Plasma and Urine", Journal of Chromatography, Biomedical Applications, 383, 1986, pp 400-404.

14. Snyder, L.R., Kirkland, J.J., Introduction to Modern Liquid Chromatography, 2nd Edition, 1979.

15. Jacob, S., Herschler, R., Knowles, R., "Proceedings on the Symposium on Dimethyl Sulfoxide", Vet. Med. Small Anim. Clin., 77, pp 365-373, March 1982.

16. Williams, K.I., Burnstein, S.H. and Layne, D.S. Proc. Soc. Exp. Biol. Med., 122: pp 865-866 (1966), Arch. Biochem. Biophys., 113, pp 251-252 (1966).

17. Hawk's Physiological Chemistry, 14th Edition, pp 1153-1205, 1965 McGraw-Hill Inc.

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